



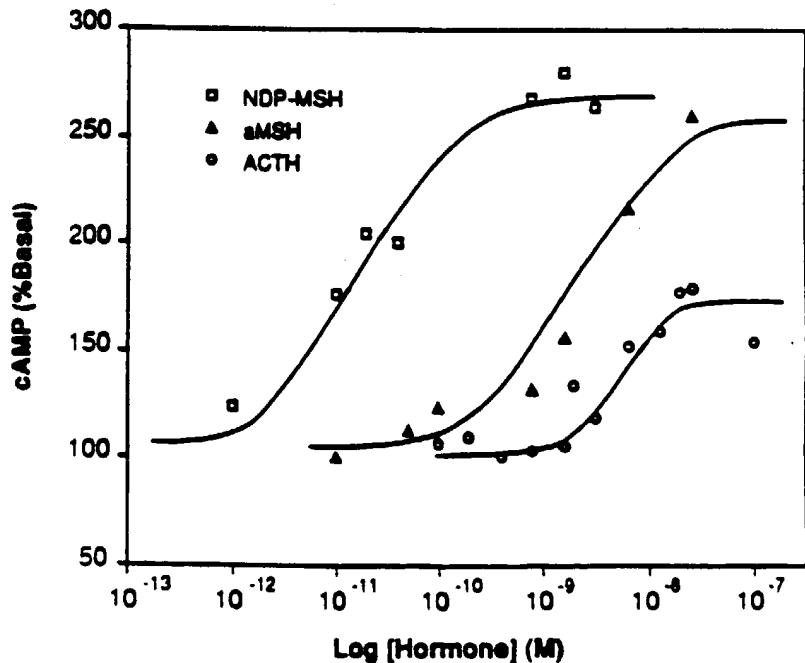
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(21) International Application Number: PCT/US93/03247		(74) Agent: NOONAN, Kevin, E.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).
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(71) Applicant: STATE OF OREGON, acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION on behalf of THE OREGON HEALTH SCIENCES UNIVERSITY [US/US]; 3181 S.W. Sam Jackson Park Road, Portland, OR 97201-3098 (US).		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(72) Inventors: CONE, Roger, D. ; 16563 S. Hatton Road, Oregon City, OR 97045 (US). MOUNTJOY, Kathleen, G. ; 301 SW Lincoln, #610, Portland, OR 97201 (US).		

## (54) Title: MAMMALIAN MELANOCYTE STIMULATING HORMONE RECEPTORS AND USES

## (57) Abstract

The present invention relates to a mammalian melanocyte stimulating hormone receptor. The invention is directed toward the isolation, characterization and pharmacological use of mammalian melanocyte stimulating hormone receptor, the gene corresponding to this receptor, a recombinant eukaryotic expression construct capable of expressing a mammalian melanocyte stimulating hormone receptor in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize mammalian melanocyte stimulating hormone receptor. The invention also provides methods for screening MSH<sup>R</sup> agonists and antagonists *in vitro* using preparations of receptor from such cultures of eukaryotic cells transformed with a recombinant eukaryotic expression construct comprising the MSH<sup>R</sup> receptor gene. The invention specifically provides human and mouse MSH<sup>R</sup> genes.



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## MAMMALIAN MELANOCYTE STIMULATING HORMONE RECEPTORS AND USES

### BACKGROUND OF THE INVENTION

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This invention was made with government support under 1R01DK41921-03, 1R01DK43859-01, and 1P01DK44239-10A1 by the National Institutes of Health. The government has certain rights in the invention.

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#### 1. Field of the Invention

This invention relates to melanocyte stimulating hormone receptors from mammalian species and the genes corresponding to such receptors. Specifically, the invention relates to the isolation, cloning and sequencing of a human melanocyte stimulating hormone receptor gene. The invention also relates to the isolation, cloning and sequencing of a mouse melanocyte stimulating hormone receptor gene. The invention relates to the construction of eukaryotic recombinant expression constructs capable of expressing these melanocyte stimulating hormone receptors in cultures of transformed eukaryotic cells, and the production of the melanocyte stimulating hormone receptor in such cultures. The invention relates to the use of such cultures of transformed eukaryotic cells to produce homogeneous compositions of such melanocyte stimulating hormone receptors. The invention also provides cultures of such cells producing melanocyte stimulating hormone receptor for the characterization of novel and useful drugs. Antibodies against and epitopes of these melanocyte stimulating hormone receptor proteins are also provided by the invention.

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#### 2. Background of the Invention

The proopiomelanocortin (POMC) gene product is processed to produce a large number of biologically active peptides. Two of these peptides,  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ MSH), and adrenocorticotrophic hormone (ACTH) have well understood roles in control of melanocyte and adrenocortical function, respectively. Both of these hormones, however, are found in a variety of forms with unknown functions. The melanocortin peptides also have a diverse

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array of biological activities in other tissues, including the brain, and immune system, and bind to specific receptors there with a distinct pharmacology [see, Hanneman *et al.*, in *Peptide Hormone as Prohormones*, G. Martinez, ed. (Ellis Horwood Ltd.: Chichester, UK) pp. 53-82; DeWied & Jolles, 1982, *Physiol. Rev.* 62: 976-1059 for reviews].

5 A complete understanding of these peptides and their diverse biological activities requires the isolation and characterization of their corresponding receptors. Some biochemical studies have been reported on the prior art.

Shimuze, 1985, *Yale J. Biol. Med.* 58: 561-570 discusses the physiology 10 of melanocyte stimulating hormone.

Tatro & Reichlin, 1987, *Endocrinology* 121: 1900-1907 disclose that MSH receptors are widely distributed in rodent tissues.

15 Solca *et al.*, 1989, *J. Biol. Chem.* 264: 14277-14280 disclose the molecular weight characterization of mouse and human MSH receptors linked to radioactively and photoaffinity labeled MSH analogues.

Siegrist *et al.*, 1991, *J. Receptor Res.* 11: 323-331 disclose the quantification of receptors on mouse melanoma tissue by receptor autoradiography.

20 The present invention comprises a human melanocyte stimulating hormone receptor gene, the nucleotide sequence of this gene and the deduced amino acid sequence of its cognate protein, a homogeneous composition of the melanocyte stimulating hormone receptor, nucleic acid hybridization probes and a method for determining the tissue distribution of expression of the gene, a recombinant expression construct capable of expressing the gene in cultures of transformed eukaryotic cells, and such cultures of transformed eukaryotic cells useful in the 25 characterization of novel and useful drugs. The present invention also comprises the homologue of the human melanocyte stimulating hormone receptor gene from the mouse.

**DESCRIPTION OF THE DRAWINGS**

Figure 1 illustrates the nucleotide sequence of the mouse (SEQ ID NO:3) and human (SEQ ID NO:5) melanocyte stimulating hormone receptor.

5 Figure 2 presents an amino acid sequence comparison between the mouse and human melanocyte stimulating hormone receptor proteins.

Figure 3 illustrates binding of melanocyte stimulating hormone receptor agonists to mouse melanocyte stimulating hormone receptor expressed in human 293 cells.

10 Figure 4 illustrates the tissue distribution of human (Panel A) and mouse (Panel B) melanocyte stimulating hormone receptor gene expression by Northern blot hybridization.

## SUMMARY OF THE INVENTION

The present invention relates to the cloning, expression and functional characterization of mammalian melanocyte stimulating hormone receptor (MSH<sup>R</sup>) genes. The invention comprises the nucleotide sequence of these genes encoding the mammalian MSH<sup>R</sup>s and the deduced amino acid sequences of the cognate proteins, as well as tissue distribution patterns of expression of these genes.

In particular, the present invention is directed toward the isolation, characterization and pharmacological use of the human MSH<sup>R</sup>, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the human MSH<sup>R</sup>, a recombinant eukaryotic expression construct capable of expressing the human MSH<sup>R</sup> in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the human MSH<sup>R</sup>, a homogeneous composition of the human MSH<sup>R</sup>, and antibodies against and epitopes of the human MSH<sup>R</sup>.

The present invention is also directed toward the isolation, characterization and pharmacological use of the mouse MSH<sup>R</sup>, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the mouse MSH<sup>R</sup>, a recombinant eukaryotic expression construct capable of expressing the mouse MSH<sup>R</sup> in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the mouse MSH<sup>R</sup>, a homogeneous composition of the mouse MSH<sup>R</sup>, and antibodies against and epitopes of the mouse MSH<sup>R</sup>.

It is an object of the invention to provide a nucleic acid comprising a nucleotide sequence encoding a mammalian MSH<sup>R</sup>. In a preferred embodiment of the invention, the nucleotide sequence encodes the human MSH<sup>R</sup>. In another preferred embodiment, the nucleotide sequence encodes the mouse MSH<sup>R</sup>.

The present invention includes a nucleic acid comprising a nucleotide sequence encoding a human MSH<sup>R</sup> receptor derived from a DNA molecule isolated from a human genomic library (SEQ ID NO:5). In this embodiment of the invention, the nucleotide sequence includes 1635 nucleotides of the human MSH<sup>R</sup> gene comprising 953 nucleotides of coding sequence, 462 nucleotides of

5' untranslated sequence and 220 nucleotides of 3' untranslated sequence.

The present invention also includes a nucleic acid comprising a nucleotide sequence encoding a mouse MSH<sup>R</sup> derived from a cDNA molecule isolated from a CDNA library constructed with RNA from mouse Cloudman melanoma cells (SEQ ID NO:3). In this embodiment of the invention, the nucleotide sequence includes 1260 nucleotides of the mouse MSH<sup>R</sup> gene comprising 947 nucleotides of coding sequence, 15 nucleotides of 5' untranslated sequence and 298 nucleotides of 3' untranslated sequence.

10 The invention includes nucleic acids comprising the nucleotide sequences of mammalian MSH<sup>R</sup>'s, most preferably mouse and human MSH<sup>R</sup>'s (SEQ ID NOs:3&5), and includes allelic variations of these nucleotide sequences and the corresponding MSH<sup>R</sup> molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, each such variant having essentially the same nucleotide sequence as the nucleotide sequence of the corresponding MSH<sup>R</sup> disclosed herein, wherein the resulting MSH<sup>R</sup> molecule has substantially the same biological properties as the MSH<sup>R</sup> molecule corresponding to the nucleotide sequence described herein. The term "substantially homologous to" as used in this invention encompasses such allelic variability as described in this paragraph.

15 The invention also includes a protein comprised of a predicted amino acid sequence for the mouse (SEQ ID NO:4) and human (SEQ ID NO:6) MSH<sup>R</sup> deduced from the nucleotide sequence comprising the complete coding sequence of the mouse (SEQ ID NO:3) and human (SEQ ID NO:5) MSH<sup>R</sup> gene as described herein.

20 In another aspect, the invention comprises a homogeneous composition of a 35.3 kilodalton mouse MSH<sup>R</sup> or derivative thereof, wherein the amino acid sequence of the MSH<sup>R</sup> or derivative thereof comprises the mouse MSH-R sequence shown in Figure 2 (SEQ ID NO:4).

25 In another aspect, the invention comprises a homogeneous composition of a 34.7 kilodalton human MSH<sup>R</sup> or derivative thereof, wherein the amino acid sequence of the MSH<sup>R</sup> or derivative thereof comprises the human MSH-R sequence shown in Figure 2 (SEQ ID NO:6).

This invention provides both nucleotide and amino acid probes derived from these sequences. The invention includes probes isolated from either cDNA or genomic DNA clones, as well as probes made synthetically with the sequence information derived therefrom. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or *in vitro* amplified probes made using cDNA or genomic clone embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the nucleotide sequence information of cDNA or genomic clone embodiments of the invention.

5 It is a further object of this invention to provide sequences of mammalian MSH<sup>R</sup>, preferably the mouse or human MSH<sup>R</sup>, for use as nucleic acid hybridization probes to determine the pattern, amount and extent of expression of this receptor in various tissues of mammals, including humans. It is also an object of the present invention to provide nucleic acid hybridization probes derived from the sequences of the mouse or human MSH<sup>R</sup> to be used for the detection and diagnosis of genetic diseases. It is an object of this invention to provide nucleic acid hybridization probes derived from the DNA sequences of the mouse or human MSH<sup>R</sup> to be used for the detection of novel related receptor genes.

10 15 20 The present invention also includes synthetic peptides made using the nucleotide sequence information comprising cDNA or genomic clone embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of MSH<sup>R</sup>-specific antibodies, or used for competitors of the MSH<sup>R</sup> molecule for drug binding, or to be used for the production of inhibitors of the binding of agonists or antagonists 25 or analogues thereof to MSH<sup>R</sup> molecule.

25 30 The present invention also provides antibodies against and epitopes of mammalian MSH<sup>R</sup>'s, preferably mouse or human MSH<sup>R</sup> proteins. It is an object of the present invention to provide antibodies that is immunologically reactive to a mammalian MSH<sup>R</sup> protein. It is a particular object of the invention to provide a monoclonal antibodies to mammalian MSH<sup>R</sup> protein, most preferably mouse or

human MSH<sup>R</sup> protein.

It is also an object of the present invention to provide a hybridoma cell line that produces such an antibody. It is a particular object of the invention to provide a hybridoma cell line that is the result of fusion between a non-immunoglobulin producing mouse myeloma cell line and spleen cells derived from a mouse immunized with a human cell line which expresses MSH<sup>R</sup> antigen. The present invention also provides a hybridoma cell line that produces such an antibody, and that can be injected into a living mouse to provide an ascites fluid from the mouse that is comprised of such an antibody.

10 The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody that is immunologically reactive to a mammalian MSH<sup>R</sup>, preferably a mouse or human MSH<sup>R</sup>, and in a pharmaceutically acceptable carrier.

15 It is a further object of the present invention to provide an epitope of a mammalian MSH<sup>R</sup> protein wherein the epitope is immunologically reactive to an antibody specific for the mammalian MSH<sup>R</sup>. In preferred embodiments, the epitope is derived from mouse or human MSH<sup>R</sup> protein.

20 It is another object of the invention to provide a chimeric antibody that is immunologically reactive to a mammalian MSH<sup>R</sup> protein. In a preferred embodiment, the chimeric antibody is a monoclonal antibody. In a preferred embodiment, the MSH<sup>R</sup> is a mouse or human MSH<sup>R</sup>.

25 The present invention provides a recombinant expression construct comprising the nucleotide sequence of a mammalian MSH<sup>R</sup>, preferably the mouse or human MSH<sup>R</sup> and sequences sufficient to direct the synthesis of mouse or human MSH<sup>R</sup> in cultures of transformed eukaryotic cells. In a preferred embodiment, the recombinant expression construct is comprised of plasmid sequences derived from the plasmid pcDNAI/neo and cDNA or genomic DNA of mouse or human MSH<sup>R</sup> gene. This invention includes a recombinant expression construct comprising essentially the nucleotide sequences of genomic or cDNA clones of mouse or human MSH<sup>R</sup> in an embodiment that provides for their expression in cultures of transformed eukaryotic cells.

It is also an object of this invention to provide cultures of transformed eukaryotic cells that have been transformed with such a recombinant expression construct and that synthesize mammalian, preferably mouse or human, MSH<sup>R</sup> protein. In a preferred embodiment, the invention provides human 293 cells that synthesize mouse MSH<sup>R</sup>. In an additional preferred embodiment, the invention provides human 293 cells that synthesize human MSH<sup>R</sup> protein.

The present invention also includes protein preparations of mammalian, preferably mouse or human MSH<sup>R</sup>, and preparations of membranes containing mammalian MSH<sup>R</sup>, derived from cultures of transformed eukaryotic cells. In a preferred embodiment, cell membranes containing mouse MSH<sup>R</sup> protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of mouse MSH<sup>R</sup>. In another preferred embodiment, cell membranes containing human MSH<sup>R</sup> protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of human MSH<sup>R</sup>. It is also an object of this invention to provide mammalian, preferably mouse or human MSH<sup>R</sup> for use in the *in vitro* screening of novel adenosine agonist and antagonist compounds. In a preferred embodiment, membrane preparations containing the mouse MSH<sup>R</sup>, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. In another preferred embodiment, membrane preparations containing the human MSH<sup>R</sup>, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. These properties are then used to characterize such novel compounds by comparison to the binding properties of known mouse or human MSH<sup>R</sup> agonists and antagonists.

The present invention will also be useful for the *in vivo* detection of analogues of agonists or antagonists of MSH<sup>R</sup>, known or unknown, either naturally occurring or as the embodiments of a drug.

It is an object of the present invention to provide a method for the quantitative detection of agonists or antagonists, or analogues thereof, of MSH<sup>R</sup>,

known or unknown, either naturally occurring or as the embodiments of a drug. It is an additional object of the invention to provide a method to detect such agonists, antagonists, or analogues thereof in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, or any other bodily fluid.

5 Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "melanocyte stimulating hormone receptor" as used herein refers to proteins substantially homologous to, and having substantially the same biological activity as, the protein coded for by the nucleotide sequence depicted in Figure 1 (SEQ ID NO:3). This definition is intended to encompass natural allelic variations in the melanocyte stimulating hormone receptor sequence. Cloned genes of the present invention may code for MSH<sup>R</sup>s of any species of origin, including, for example, mouse, rat, rabbit, cat, and human, but preferably code for receptors of mammalian, most preferably mouse and human, origin.

Nucleic acid hybridization probes provided by the invention comprise DNA sequences that are substantially homologous to the DNA sequences in Figure 1A (SEQ ID NO:3) and 1B (SEQ ID NO:5). Nucleic acid probes are useful for detecting MSH<sup>R</sup> gene expression in cells and tissues using techniques well-known in the art, including but not limited to Northern blot hybridization, *in situ* hybridization and Southern hybridization to reverse transcriptase - polymerase chain reaction product DNAs. The probes provided by the present invention, including oligonucleotides probes derived therefrom, are useful are also useful for Southern hybridization of mammalian, preferably human, genomic DNA for screening for restriction fragment length polymorphism (RFLP) associated with certain genetic disorders.

The production of proteins such as the MSH<sup>R</sup> from cloned genes by genetic engineering is well known. See, e.g., U.S. Patent No. 4,761,371 to Bell *et al.* at Col. 6 line 3 to Col. 9 line 65. (The disclosure of all U.S. patent references cited herein is to be incorporated herein by reference.) The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

DNA which encodes the MSH<sup>R</sup> may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide

probes generated from the MSH<sup>R</sup> gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, MSH<sup>R</sup> gene sequences may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the MSH<sup>R</sup> gene sequence provided herein. See U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis.

The MSH<sup>R</sup> may be synthesized in host cells transformed with a recombinant expression construct comprising a DNA sequence encoding the MSH<sup>R</sup>. Such a recombinant expression construct can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding the MSH<sup>R</sup> and/or to express DNA which encodes the MSH<sup>R</sup>. For the purposes of this invention, a recombinant expression construct is a replicable DNA construct in which a DNA sequence encoding the MSH<sup>R</sup> is operably linked to suitable control sequences capable of effecting the expression of the MSH<sup>R</sup> in a suitable host. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the

intended expression host. A preferred vector is the plasmid pcDNA1/neo. Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising a mammalian MSH<sup>R</sup>. Transformed host cells may ordinarily express the mammalian MSH<sup>R</sup>, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridization probe DNA need not express the receptor. When expressed, the mammalian MSH<sup>R</sup> will typically be located in the host cell membrane.

DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leaders sequences, contiguous and in the same translational reading frame.

Cultures of cells derived from multicellular organisms are a desirable host for recombinant MSH<sup>R</sup> synthesis. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure. See Tissue Culture, Academic Press, Kruse & Patterson, editors (1973). Examples of useful host cell lines are human 293 cells, VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI38, BHK, COS-7, CV, and MDCK cell lines. Human 293 cells are preferred. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream from the gene to be expressed, along with a ribosome binding site, RNA splice sites (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral source (e.g., polyoma, adenovirus, VSV, or MPV), or may be provided by the host cell chromosomal replication mechanism. If the vector is

integrated into the host cell chromosome, the latter may be sufficient.

The invention provides homogeneous compositions of mammalian MSH<sup>R</sup> protein produced by transformed eukaryotic cells as provided herein. Such homogeneous compositions are intended to be comprised of mammalian MSH<sup>R</sup> protein that comprises 90% of the protein in such homogenous composition.

Mammalian MSH<sup>R</sup> protein made from cloned genes in accordance with the present invention may be used for screening agonist compounds for MSH<sup>R</sup> activity, or for determining the amount of a MSH<sup>R</sup> agonist or antagonist drug in a solution (e.g., blood plasma or serum). For example, host cells may be transformed with a recombinant expression construct of the present invention, MSH<sup>R</sup> expressed in that host, the cells lysed, and the membranes from those cells used to screen compounds for MSH<sup>R</sup> binding activity. Competitive binding assays in which such procedures may be carried out are well known in the art. By selection of host cells which do not ordinarily express MSH<sup>R</sup>s, pure preparations of membranes containing MSH<sup>R</sup>s can be obtained. Further, MSH<sup>R</sup> agonists and antagonists can be identified by transforming host cells with vectors of the present invention. Membranes obtained from such cells can be used in binding studies wherein the drug dissociation activity is monitored.

The recombinant expression constructs of the present invention are useful in molecular biology to transform cells which do not ordinarily express the MSH<sup>R</sup> to thereafter express this receptor. Such cells are useful as intermediates for making cell membrane preparations useful for receptor binding assays, which are in turn useful for drug screening. Further, genes and vectors comprising the recombinant expression construct of the present invention are useful in gene therapy. For such purposes, retroviral vectors as described in U.S. Patent No. 4,650,764 to Temin & Watanabe or U.S. Patent No. 4,861,719 to Miller may be employed. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out homologous recombination or site-directed mutagenesis. *See generally* Thomas & Capecchi, 1987, *Cell* 51: 503-512; Bertling, 1987, *Bioscience Reports* 7: 107-112; Smithies *et al.*, 1985, *Nature* 317: 230-234.

Oligonucleotides of the present invention are useful as diagnostic tools for probing MSH receptor gene expression in tissues. For example, tissues can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence of the MSH<sup>R</sup> gene, and potential pathological conditions related thereto, as also illustrated by the Examples below.

The invention also provides antibodies that are immunologically reactive to a mammalian MSH<sup>R</sup>. The antibodies provided by the invention can be raised in animals by inoculation with cells that express a mammalian MSH<sup>R</sup> or epitopes of a mammalian MSH<sup>R</sup> using methods well known in the art. Animals that can be used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals for inoculation are rodents (including mice, rats, hamsters) and rabbits. The most preferred animal is the mouse.

Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell line which naturally expresses a mammalian MSH<sup>R</sup>, or any cell or cell line that expresses a mammalian MSH<sup>R</sup> or any epitope therein as a result of molecular or genetic engineering, or that has been treated to increase the expression of a mammalian MSH<sup>R</sup> by physical, biochemical or genetic means. Preferred cells are human cells, most preferably human 293 cells that have been transformed with a recombinant expression construct comprising DNA sequences encoding a mammalian MSH<sup>R</sup> and that express the mammalian MSH<sup>R</sup> gene product.

The present invention provides monoclonal antibodies that are immunologically reactive with an epitope that is a mammalian MSH<sup>R</sup> present on the surface of mammalian cells, preferably human or mouse cells. These antibodies are made using methods and techniques well known to those of skill in the art.

Monoclonal antibodies provided by the present invention are produced by

hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art. Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with cells expressing a mammalian MSH<sup>R</sup>, including human cells, as described above. The myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from mouse, and the most preferred mouse myeloma cell line is P3X63-Ag8.653. The animals from whom spleens are obtained after immunization are rats, mice and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from *in vitro* cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a mouse, and the monoclonal antibodies obtained from blood and/or ascites fluid.

Monoclonal antibodies provided by the present invention can also be produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are immunologically reactive with an epitope of a mammalian MSH<sup>R</sup>.

The present invention encompasses fragments of the antibody that are immunologically reactive with an epitope of a mammalian MSH<sup>R</sup>. Such fragments can be produced by any number of methods, including but not limited to proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mammalian MSH<sup>R</sup> made by methods known to those of skill in the art.

The present invention also encompasses an epitope of a mammalian MSH<sup>R</sup> that is comprised of sequences and/or a conformation of sequences present in the

mammalian MSH<sup>R</sup> molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mammalian MSH<sup>R</sup> molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using methods well known to those skilled in the art.

5 The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered prokaryotic or eukaryotic cells.

10 The invention also includes chimeric antibodies, comprised of immunologically reactive light chain and heavy chain peptides to an epitope that is a mammalian MSH<sup>R</sup>. The chimeric antibodies embodied in the present invention include those that are derived from naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

15 The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

#### EXAMPLE 1

20 **Isolation of an  $\alpha$ MSH Receptor Probe by Random  
PCR Amplification of Human Melanoma cDNA Using  
Degenerate Oligonucleotide Primers**

25 In order to clone novel G-protein coupled receptors, human melanoma cDNA was used as template for a polymerase chain reaction (PCR)-based random cloning experiment. PCR was performed using a pair of degenerate oligonucleotide primers corresponding to the putative third and sixth transmembrane regions of G-protein coupled receptors (Libert *et al.*, 1989, *Science* **244**: 569-72; Zhou *et al.*, 1990, *Nature* **347**: 76-80). The PCR products obtained in this experiment were characterized by nucleotide sequencing. Two novel sequences representing novel G-protein-coupled receptors were identified.

30 PCR amplification was performed as follows. Total RNA was isolated from a human melanoma tumor sample by the guanidinium thiocyanate method

(Chirgwin *et al.*, 1979, *Biochemistry* **18**: 5294-5299). Double-stranded cDNA was synthesized from total RNA with murine reverse transcriptase (BRL, Gaithersburg, MD) by oligo-dT priming [Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), 1990]. The melanoma cDNA mixture was then subjected to 45 cycles of PCR amplification using 500 picomoles of degenerate oligonucleotide primers having the following sequence:

### Primer III (sense):

10

**GAGTCGACCTGTG(C/T)G(C/T)(C/G)AT(C/T)(A/G)CIIIT(G/T)GAC(C/A)G(C/G)T**  
**AC**

(SEQ ID NO:1)

15

and

### Primer VI (antisense):

2

**CAGAATTCA(GT/A)AGGGCAICCAGCAGAI(G/C)(G/A)(T/C)GAA  
(SEQ ID NO:2)**

in 100  $\mu$ l of a solution containing 50 mM Tris-HCl (pH 8.3), 2.5 mM MgCl<sub>2</sub>, 0.01% gelatin, 200  $\mu$ M each dNTP, and 2.5 units of *Taq* polymerase (Saiki *et al.*, 1988, *Science* 239: 487-491). These primers were commercially synthesized by Research Genetics Inc. (Huntsville, AL). Each PCR amplification cycle consisted of incubations at 94°C for 1 min (denaturation), 45°C for 2 min (annealing), and 72°C for 2 min (extension).

Amplified products of the PCR reaction were extracted with phenol/chloroform and precipitated with ethanol. After digestion with *Eco*RI and *Sall*, the PCR products were separated on a 1.2% agarose gel. A slice of this gel, corresponding to PCR products of 300 basepairs (bp) in size, was cut out and purified using glass beads and sodium iodide, and the insert was then cloned into a pBKS cloning vector (Stratagene, LaJolla, CA).

35 A total of 172 of such pBKS clones containing inserts were sequenced using Sequenase (U. S. Biochemical Corp., Cleveland, OH) by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977, Proc. Natl. Acad. Sci. USA 74: 5463-5467). Two types of sequences homologous to other

G-protein coupled receptors were identified.

## EXAMPLE 2

5 Isolation and Sequence Analysis of Mouse  $\alpha$ MSH Receptor cDNA

Probes isolated in Example 1 was used to screen a Cloudman melanoma cDNA library in order to isolate a full-length cDNA corresponding to the cloned probe. One clone was isolated from a library of  $5 \times 10^6$  clones screened as 10 described below. This clone contained an insert of 2.6 kilobases (kb). The nucleotide sequence of the complete coding region was determined, as shown in Figure 1A (SEQ ID NO:3).

The PCR probe was labeled by the random-priming method (Stratagene PrimeIt, #300387, LaJolla, CA) and used to screen a Cloudman melanoma line cDNA library constructed in the  $\lambda$ ZAP vector (Stratagene). Library screening 15 was performed using techniques well-known in the art as described in Bunzow *et al.* (1988, *Nature* **336**: 783-787) at moderate stringency (40% formamide, 1M NaCl, 50mM Tris-HCl, pH 7.5, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 100 $\mu$ g/ml salmon sperm DNA, 10X Denhardt's solution). One 20 cDNA clone was identified (termed mmelA) and its 2.6 kb cDNA insert was isolated and subcloned into pBKS (Stratagene); the resulting plasmid was called pmmelA. Nucleotide sequence analysis and homology comparisons were done on the OHSU computer system with software provided by Intelligenetics Inc. (Mountain View, CA).

25 The nucleotide sequence of pmmelA (the cDNA clone isolated as described above) is shown in Figure 1A (SEQ ID NO:3). The longest open reading frame of this cDNA encodes a predicted protein product of 315 amino acids with a calculated molecular weight of 35.3 kilodaltons (kD). The deduced amino acid sequence is shown in Figure 2 (SEQ ID NO:4) as mouse MSH-R. Single letter 30 amino acid codes are used [see, G. Zubay, *Biochemistry* (2d ed.), 1988 (MacMillen Publishing: New York) p.33]. Uppercase lettering indicates amino acid residues in common between the receptor proteins shown; lowercase lettering indicates divergent residues.

Hydrophobicity analysis (Kyte & Doolittle, 1982, *J. Mol. Biol.* 157: 105-132) of the deduced amino acid sequence showed that the protein contains seven hydrophobic stretches of 21 to 26 amino acids apiece. Putative transmembrane domains are overlined and designated with Roman numerals.

5

### EXAMPLE 3

#### Construction of Mouse $\alpha$ MSH<sup>R</sup> Expression Plasmids, DNA Transfection and Functional Expression of the $\alpha$ MSH<sup>R</sup> Gene Product

10

In order to biochemically characterize the putative mouse  $\alpha$ MSH<sup>R</sup> cDNA isolated as in Example 2, and to confirm that it encodes an  $\alpha$ MSH receptor, mmelA was cloned into a mammalian expression vector, this vector transfected into human 293 cells, and cell lines generated that expressed the putative  $\alpha$ MSH<sup>R</sup> receptor at the cell surface. Such cells and membranes isolated from such cells were used for biochemical characterization experiments described below.

The entire coding region of the  $\alpha$ MSH<sup>R</sup> cDNA insert from mmelA contained in a 2.1kb fragment was excised from pBSK and subcloned into the *Bam*H/*Xba*I sites of pcDNA1/neo expression vector (Invitrogen, San Diego, CA). The resulting plasmid was called pcDNA-mmelA. pcDNA-mmelA plasmid DNA was prepared in large-scale through one cycle of CsCl gradient ultracentrifugation and 20  $\mu$ g pcDNA-mmelA DNA were transfected into each 100mm dish of 293 cells using the calcium phosphate method (see Chen & Okayama, 1987, *Mol. Cell. Biol.* 7: 2745-2752). After transfection, cells were cultured in DMEM media supplemented with 10% calf serum in a 3% CO<sub>2</sub> atmosphere at 37°C. Selection was performed with neomycin (G418; GIBCO) at a concentration of 1000  $\mu$ g/ml; selection was started 72 hr after transfection and continued for 3 weeks.

The  $\alpha$ MSH<sup>R</sup> is known to couple to G-proteins and thereby activate adenylyl cyclase, increasing intracellular levels of cAMP (see Buckley & Ramachandran, 1981, *Proc. Natl. Acad. Sci. USA* 78: 7431-7435; Grahame-Smith *et al.*, 1967, *J. Biol. Chem.* 242: 5535-5541; Mertz & Catt, 1991, *Proc. Natl. Acad. Sci. USA* 88: 8525-8529; Pawalek *et al.*, 1976, *Invest. Dermatol.* 66: 200-209). This

property of cells expressing the  $\alpha$ MSH receptor was used analyze expression of the  $\alpha$ MSH receptor in cell colonies transfected with the expression vectors described herein as follows. Cells ( $\sim 1 \times 10^6$ ) were plated in 6-well dishes, washed once with DMEM containing 1% bovine serum albumin (BSA) and 0.5mM IBMX (a phosphodiesterase inhibitor), then incubated for 45 minutes at 37°C with varying concentrations of the melanotropic peptides  $\alpha$ MSH,  $\beta$ MSH,  $\gamma$ MSH, the MSH peptide analogues Nle<sup>4</sup>, D-Phe<sup>7</sup>- $\alpha$ MSH (NDP-MSH), and ACTH. Following hormone treatment, the cells were washed twice with phosphate buffered saline and intracellular cAMP extracted by lysing the cells with 1ml of 60% ethanol. Intracellular cAMP concentrations were determined using an assay (Amersham) which measures the ability of cAMP to displace [ $^3$ H] cAMP from a high affinity cAMP binding protein (see Gilman, 1970, Proc. Natl. Acad. Sci. USA 67: 305-312).

The results of these experiments are shown in Figure 3. The abscissa indicates the concentration of each hormone and the ordinate indicates the percentage of basal intracellular cAMP concentration achieved by each treatment. Points indicate the mean of duplicate incubations; the standard error did not exceed 15% for any data point. None of the peptides tested induced any change in intracellular cAMP in cells containing the vector alone. Cells expressing the murine  $\alpha$ MSH receptor responded to melanotropic peptides with a 2-3 fold elevation of intracellular cAMP, similar to levels of cAMP induced by these peptides in the Cloudman cell line (see Pawalek, 1985, Yale J. Biol. Med. 58: 571-578). The EC<sub>50</sub> values determined for  $\alpha$ MSH ( $2.0 \times 10^{-9}$ M), ACTH ( $8.0 \times 10^{-9}$ M) and the superpotent MSH analogue NDP-MSH ( $2.8 \times 10^{-11}$ M) correspond closely to reported values (see Tatro *et al.*, 1990, Cancer Res. 50: 1237-1242). As expected, the  $\beta$ MSH peptide had an EC<sub>50</sub> value comparable to  $\alpha$ MSH<sup>22</sup> while  $\gamma$ MSH had little or no activity (see Slominski *et al.*, 1992, Life Sci. 50: 1103-1108), confirming the identity of this receptor as a melanocyte  $\alpha$ MSH receptor.

## EXAMPLE 4

Isolation and Characterization of a Human  $\alpha$ MSH<sup>R</sup> Genomic Clone

5        In order to isolate a human counterpart of the murine melanocyte  $\alpha$ MSH receptor gene, a human genomic library was screened at high stringency (50% formamide, 42°C) using the human PCR fragments isolated as described in Example 1. Two different types of sequences were isolated, corresponding to the two PCR fragments, and were found to encode highly related G protein-coupled receptors. These genomic clones were sequenced as described in Example 2. One of these genomic clones was determined to encode an human MSH receptor (SEQ ID NO:5). The human MSH receptor has a predicted amino acid sequence (SEQ ID NO:6) that is 75 % identical and colinear with the mouse  $\alpha$ MSH receptor cDNA sequence (Figure 2), represented as human MSH-R. The predicted molecular weight of the human MSH<sup>R</sup> is 34.7kD.

10       The predicted amino acid sequences of the mouse  $\alpha$ MSH<sup>R</sup> (SEQ ID NO:4) and human MSH<sup>R</sup> (SEQ ID NO:6) are aligned in Figure 2. These sequences define the melanocortin receptors as a novel subfamily of the G protein-coupled receptors with a number of unusual features. The melanocortin receptors are the 15       smallest G protein-coupled receptors identified to date (297-317aa) resulting from a short amino terminal extracellular domain, a short carboxy-terminal intracellular domain, and a very small third intracellular loop. The melanocortin receptors are 20       lack several amino acid residues present in most G protein coupled receptors (see Probst *et al.*, 1992, DNA & Cell Biol. 11: 1-20), including the proline residues 25       in the 4th and 5th transmembrane domains, likely to introduce a bend in the alpha helical structure of the transmembrane domains and thought to be involved in the formation of the binding pocket (see Applebury & Hargrave, 1986, Vision Res. 26: 1881-1895), and one or both of the cysteine residues thought to form a disulfide bond between the first and second extracellular loops (see Dixon *et al.*, 30       1987, EMBO J. 6: 3269-3275 and Karnik *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85: 8459-8463). Remarkably, the melanocortin receptors do not appear highly related to the other G protein-coupled receptors which recognize peptide ligands, such as the receptors for bombesin (see Spindel *et al.*, 1990, Mol.

Endocrinol. 4: 1956-1963) or substance K (see Masu *et al.*, 1987, Nature 329: 836-838) but rather, are more closely related to the receptor for  $\Delta^9$ -tetrahydrocannabinol (see Matsuda *et al.*, 1990, Nature 346: 561-564). The cannabinoid receptor also lacks the conserved proline in transmembrane 5 and the cysteine in the first extracellular loop necessary for disulfide bond formation. 5 Least parsimony analysis with the receptor sequences shown in Figure 2 suggests the cannabinoid and melanocortin receptors may be evolutionarily related and form a subfamily distinct from the peptide receptors and the amine receptors. Regardless of whether the similarities are the result of evolutionary conservation 10 or convergence, the sequence and putative structural similarities between the melanocortin and cannabinoid receptors may be informative in the search for the endogenous cannabinoid-like ligand.

#### EXAMPLE 5

15

##### Tissue Distribution of $\alpha$ MSH Receptors

To further gain insight into these receptors, we have examined the tissue distribution of their corresponding mRNAs from various tissues by performing 20 Northern hybridization experiments on RNA isolated from various tissues (see Maniatis *et al.*, *ibid.*). The results of these experiments are shown in Figure 4.

A panel of tissue samples was examined by Northern hybridization analysis 25 performed under high stringency conditions. The same nitrocellulose filter was hybridized successively with a human MSH receptor probe and a mouse MSH receptor probe to determine the distribution of each receptor mRNA. The murine MSH receptor is encoded predominantly by a single mRNA species of 3.9kb, while the human MSH receptor is encoded, in two melanoma samples, predominantly by a 3.0kb species. High levels of receptor mRNA are seen in both primary mouse melanocytes and mouse melanoma cell lines. In contrast, 30 extremely low levels of receptor mRNA were detected in primary human melanocytes, and many human melanoma samples (see melanoma 1, Fig. 4). Most intriguing is the dramatic elevation of MSH-R mRNA seen thus far in 3 of 11 samples tested, such as is seen in melanoma sample #2 (Fig. 4).

Additionally, we have been unable to detect expression in the brain of any of the receptors described here, despite extensive documentation of MSH binding sites there as well as in other tissues. These finding suggest the existence of alternate forms of these or related receptors that may be specifically expressed in  
5 brain tissue.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Cone, Roger D  
Mountjoy, Kathleen G

(ii) TITLE OF INVENTION: Melanocyte Stimulating Hormone Receptor  
and Uses

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Allegretti & Witcoff, Ltd.  
(B) STREET: 10 South Wacker Drive, Suite 3000  
(C) CITY: Chicago  
(D) STATE: Illinois  
(E) COUNTRY: USA  
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: PCT/US93/03247  
(B) FILING DATE: 07-APR-1993  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Noonan, Kevin E  
(B) REGISTRATION NUMBER: 35,303  
(C) REFERENCE/DOCKET NUMBER: 92,154-A

(ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 312-715-1000  
(B) TELEFAX: 312-715-1234  
(C) TELEX: 910-221-5317

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:  
(A) NAME/KEY: misc\_feature

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-25-

- (B) LOCATION: 1..33
- (D) OTHER INFORMATION: /function= "Degenerate oligonucleotide primer (sense)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGTCGACCT GTGYGYSATY RCTKGACMGS TAC

33

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..31
- (D) OTHER INFORMATION: /function= "Degenerate oligonucleotide primer (antisense)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGAATTCAAG WAGGGCACCA GCAGASRYGA A

31

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1260 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 15..959

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..14

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 960..1260

-26-

Asn Ala Thr Ser His Leu Gly Leu Ala Thr Asn Gln Ser Glu Pro Trp		
20	25	30
Cys Leu Tyr Val Ser Ile Pro Asp Gly Leu Phe Leu Ser Leu Gly Leu		
35	40	45
Val Ser Leu Val Glu Asn Val Leu Val Val Ile Ala Ile Thr Lys Asn		
50	55	60
Arg Asn Leu His Ser Pro Met Tyr Tyr Phe Ile Cys Cys Leu Ala Leu		
65	70	75
Ser Asp Leu Met Val Ser Val Ser Ile Val Leu Glu Thr Thr Ile Ile		
85	90	95
Leu Leu Leu Glu Val Gly Ile Leu Val Ala Arg Val Ala Leu Val Gln		
100	105	110
Gln Leu Asp Asn Leu Ile Asp Val Leu Ile Cys Gly Ser Met Val Ser		
115	120	125
Ser Leu Cys Phe Leu Gly Ile Ile Ala Ile Asp Arg Tyr Ile Ser Ile		
130	135	140
Phe Tyr Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Arg		
145	150	155
Arg Ala Val Val Gly Ile Trp Met Val Ser Ile Val Ser Ser Thr Leu		
165	170	175
Phe Ile Thr Tyr Tyr Lys His Thr Ala Val Leu Leu Cys Leu Val Thr		
180	185	190
Phe Phe Leu Ala Met Leu Ala Leu Met Ala Ile Leu Tyr Ala His Met		
195	200	205
Phe Thr Arg Ala Cys Gln His Val Gln Gly Ile Ala Gln Leu His Lys		
210	215	220
Arg Arg Arg Ser Ile Arg Gln Gly Phe Cys Leu Lys Gly Ala Ala Thr		
225	230	235
Leu Thr Ile Leu Leu Gly Ile Phe Phe Leu Cys Trp Gly Pro Phe Phe		
245	250	255
Leu His Leu Leu Leu Ile Val Leu Cys Pro Gln His Pro Thr Cys Ser		
260	265	270
Cys Ile Phe Lys Asn Phe Asn Leu Phe Leu Leu Ile Val Leu Ser		
275	280	285
Ser Thr Val Asp Pro Leu Ile Tyr Ala Phe Arg Ser Gln Glu Leu Arg		
290	295	300

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Met Thr Leu Lys Glu Val Leu Leu Cys Ser Trp  
 305 310 315

## (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1633 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 462..1415

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..461

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 1416..1633

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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AGGACGGTCC	AGAGGTGTG	AAATGTCTG	GGAACCTGAG	CAGCAGCCAC	CAGGGAAGAG	180
GCAGGGAGGG	AGCTGAGGAC	CAGGCTTGGT	TGTGAGAACATC	CCTGAGCCCA	GGCGGTTGAT	240
GCCAGGAGGT	GTCTGGACTG	GCTGGGCCAT	GCCTGGGCTG	ACCTGTCCAG	CCAGGGAGAG	300
GGTGTGAGGG	CAGATCTGGG	GGTGCCCCAGA	TGGAAGGAGG	CAGGCATGGG	GACACCCAAG	360
GCCCCCTGGC	AGCACCATGA	ACTAACCGAGG	ACACCTGGAG	GGGAAGAACT	GTGGGGACCT	420
GGAGGCCTCC	AACGACTCCT	TCCTGCTTCC	TGGACAGGAC	T ATG GCT GTG CAG		473
				Met Ala Val Gln		
				1		
GGA TCC CAG AGA AGA	CTT CTG GGC TCC	CTC AAC TCC ACC CCC ACA	GCC			521
Gly Ser Gln Arg Arg	Leu Leu Gly Ser	Leu Asn Ser Thr Pro	Thr Ala			
5	10	15	20			
ATC CCC CAG CTG GGG CTG GCT GCC AAC CAG ACA	GGA GCC CGG TGC CTG					569
Ile Pro Gln Leu Gly Leu Ala Ala Asn Gln Thr	Gly Ala Arg Cys Leu					
25	30	35				

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GAG GTG TCC ATC TCT GAC GGG CTC TTC CTC AGC CTG GGG CTG GTG AGC	617																																																																																																		
Glu Val Ser Ile Ser Asp Gly Leu Phe Leu Ser Leu Gly Leu Val Ser																																																																																																			
40	45	50		TTG CTG GAG AAC GCG CTG GTG GTG GCC ACC ATC GCC AAG AAC CGG AAC	665	Leu Val Glu Asn Ala Leu Val Val Ala Thr Ile Ala Lys Asn Arg Asn		55	60	65		CTG CAC TCA CCC ATG TAC TGC TTC ATC TGC TGC CTG GCC TTG TCG GAC	713	Leu His Ser Pro Met Tyr Cys Phe Ile Cys Cys Leu Ala Leu Ser Asp		70	75	80		CTG CTG GTG AGC GGG ACG AAC GTG CTG GAG ACG GCC GTC ATC CTC CTG	761	Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala Val Ile Leu Leu		85	90	95	100	CTG GAG CCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG	809	Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val Leu Gln Gln Leu		105	110	115		GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC	857	Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu		120	125	130		TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905	Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr		135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240	
50																																																																																																			
TTG CTG GAG AAC GCG CTG GTG GTG GCC ACC ATC GCC AAG AAC CGG AAC	665																																																																																																		
Leu Val Glu Asn Ala Leu Val Val Ala Thr Ile Ala Lys Asn Arg Asn																																																																																																			
55	60	65		CTG CAC TCA CCC ATG TAC TGC TTC ATC TGC TGC CTG GCC TTG TCG GAC	713	Leu His Ser Pro Met Tyr Cys Phe Ile Cys Cys Leu Ala Leu Ser Asp		70	75	80		CTG CTG GTG AGC GGG ACG AAC GTG CTG GAG ACG GCC GTC ATC CTC CTG	761	Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala Val Ile Leu Leu		85	90	95	100	CTG GAG CCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG	809	Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val Leu Gln Gln Leu		105	110	115		GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC	857	Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu		120	125	130		TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905	Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr		135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240									
65																																																																																																			
CTG CAC TCA CCC ATG TAC TGC TTC ATC TGC TGC CTG GCC TTG TCG GAC	713																																																																																																		
Leu His Ser Pro Met Tyr Cys Phe Ile Cys Cys Leu Ala Leu Ser Asp																																																																																																			
70	75	80		CTG CTG GTG AGC GGG ACG AAC GTG CTG GAG ACG GCC GTC ATC CTC CTG	761	Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala Val Ile Leu Leu		85	90	95	100	CTG GAG CCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG	809	Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val Leu Gln Gln Leu		105	110	115		GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC	857	Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu		120	125	130		TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905	Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr		135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																	
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CTG CTG GTG AGC GGG ACG AAC GTG CTG GAG ACG GCC GTC ATC CTC CTG	761																																																																																																		
Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala Val Ile Leu Leu																																																																																																			
85	90	95	100	CTG GAG CCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG	809	Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val Leu Gln Gln Leu		105	110	115		GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC	857	Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu		120	125	130		TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905	Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr		135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																									
95	100																																																																																																		
CTG GAG CCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG	809																																																																																																		
Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val Leu Gln Gln Leu																																																																																																			
105	110	115		GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC	857	Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu		120	125	130		TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905	Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr		135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																	
115																																																																																																			
GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC	857																																																																																																		
Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu																																																																																																			
120	125	130		TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905	Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr		135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																									
130																																																																																																			
TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC	905																																																																																																		
Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr																																																																																																			
135	140	145		GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953	Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala		150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																																	
145																																																																																																			
GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CGG GCG CCG CGA GCC	953																																																																																																		
Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala																																																																																																			
150	155	160		GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001	Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile		165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																																									
160																																																																																																			
GTT GCG GCC ATC TCG GTG GCC AGT GTC GTC TTC AGC ACG CTC TTC ATC	1001																																																																																																		
Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile																																																																																																			
165	170	175	180	CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049	Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe		185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																																																	
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CCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC	1049																																																																																																		
Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe																																																																																																			
185	190	195		CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097	Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala		200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																																																									
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CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC	1097																																																																																																		
Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val His Met Leu Ala																																																																																																			
200	205	210		CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145	Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln		215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																																																																	
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CGG GCC TCC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG	1145																																																																																																		
Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln																																																																																																			
215	220	225		CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193	Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr		230	235	240																																																																																									
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CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC	1193																																																																																																		
Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr																																																																																																			
230	235	240																																																																																																	
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ATC CTG CTG GGC ATT TTC TTC CTC TGC TGG GGC CCC TTC TTC CTG CAT Ile Leu Leu Gly Ile Phe Phe Leu Cys Trp Gly Pro Phe Phe Leu His 245 250 255 260	1241
CTC ACA CTC ATC GTC CTC TGC CCC GAG CAC CCC ACG TGC GGC TGC ATC Leu Thr Leu Ile Val Leu Cys Pro Glu His Pro Thr Cys Gly Cys Ile 265 270 275	1289
TTC AAG AAC TTC AAC CTC TTT CTC GCC CTC ATC ATC TGC AAT GCC ATC Phe Lys Asn Phe Asn Leu Phe Leu Ala Leu Ile Ile Cys Asn Ala Ile 280 285 290	1337
ATC GAC CCC CTC ATC TAC GCC TTC CAC AGC CAG GAG CTC CGC AGG ACG Ile Asp Pro Leu Ile Tyr Ala Phe His Ser Gln Glu Leu Arg Arg Thr 295 300 305	1385
CTC AAG GAG GTG CTG ACA TGC TCC TGG TGAGCGCGGT GCACGCGCTT Leu Lys Glu Val Leu Thr Cys Ser Trp 310 315	1432
TAAGTGTGCT GGGCAGAGGG AGGTGGTGAT ATTGTGGTCT GGTTCTGTG TGACCCCTGGG CAGTTCCCTTA CCTCCCTGGT CCCCCTTGT CAAAGAGGAT GGACTAAATG ATCTCTGAAA CTGTTGAAGC GCGGACCCCTT CTGGGCAGGG AGGGGTCTG CAAAACCTCA GGCAGGACTT CTCACCCAGCA GTCGTGGGAA C	1492 1552 1612 1633

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 317 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Val Gln Gly Ser Gln Arg Arg Leu Leu Gly Ser Leu Asn Ser 1 5 10 15	
Thr Pro Thr Ala Ile Pro Gln Leu Gly Leu Ala Ala Asn Gln Thr Gly 20 25 30	
Ala Arg Cys Leu Glu Val Ser Ile Ser Asp Gly Leu Phe Leu Ser Leu 35 40 45	
Gly Leu Val Ser Leu Val Glu Asn Ala Leu Val Val Ala Thr Ile Ala 50 55 60	
Lys Asn Arg Asn Leu His Ser Pro Met Tyr Cys Phe Ile Cys Cys Leu 65 70 75 80	

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Ala Leu Ser Asp Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala  
85 90 95

Val Ile Leu Leu Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val  
100 105 110

Leu Gln Gln Leu Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met  
115 120 125

Leu Ser Ser Leu Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile  
130 135 140

Ser Ile Phe Tyr Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg  
145 150 155 160

Ala Pro Arg Ala Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser  
165 170 175

Thr Leu Phe Ile Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu  
180 185 190

Val Val Phe Phe Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val  
195 200 205

His Met Leu Ala Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu  
210 215 220

His Lys Arg Gln Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala  
225 230 235 240

Val Thr Leu Thr Ile Leu Leu Gly Ile Phe Phe Leu Cys Trp Gly Pro  
245 250 255

Phe Phe Leu His Leu Thr Leu Ile Val Leu Cys Pro Glu His Pro Thr  
260 265 270

Cys Gly Cys Ile Phe Lys Asn Phe Asn Leu Phe Leu Ala Leu Ile Ile  
275 280 285

Cys Asn Ala Ile Ile Asp Pro Leu Ile Tyr Ala Phe His Ser Gln Glu  
290 295 300

Leu Arg Arg Thr Leu Lys Glu Val Leu Thr Cys Ser Trp  
305 310 315

SUBSTITUTE SHEET

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WHAT WE CLAIM IS:

1. A nucleic acid comprising a nucleotide sequence encoding a mammalian melanocyte stimulating hormone receptor.
2. A nucleic acid according to Claim 1 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.
3. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1A (SEQ ID NO:3).
4. A nucleic acid according to Claim 1 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.
105. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1B (SEQ ID NO:5).
6. A DNA sequence according to Claim 1 wherein the mammalian melanocyte stimulating hormone receptor encoded therein has the melanotropic peptide response properties described in Figure 3.
157. A homogeneous composition of a 35.3 kilodalton melanocyte stimulating hormone receptor or derivative thereof, wherein the amino acid sequence of the melanocyte stimulating hormone receptor or derivative thereof comprises the mouse MSH-R sequence shown in Figure 2 (SEQ ID NO:4).
8. A homogeneous composition of a 34.6 kilodalton melanocyte stimulating hormone receptor or derivative thereof, wherein the amino acid sequence of the melanocyte stimulating hormone receptor or derivative thereof comprises the human MSH-R sequence shown in Figure 2 (SEQ ID NO:6).
9. A nucleic acid hybridization probe for the detection of mammalian melanocyte stimulating hormone receptor expression comprising the nucleotide sequence of Claim 3.
10. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a human.
11. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.
12. A nucleic acid hybridization probe for the detection of mammalian melanocyte stimulating hormone receptor expression comprising the nucleotide sequence of Claim 5.
- 35 13. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a

human.

14. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.

15. A recombinant expression construct comprising a nucleotide sequence encoding a mammalian melanocyte stimulating hormone receptor.

16. A recombinant expression construct comprising the DNA sequence of Claim 3, wherein the construct is capable of expressing the mouse melanocyte stimulating hormone receptor in a transformed eukaryotic cell culture.

1017. A recombinant expression construct comprising the DNA sequence of Claim 5, wherein the construct is capable of expressing the human melanocyte stimulating hormone receptor in a transformed eukaryotic cell culture.

18. The recombinant expression construct of Claim 15 comprising pcDNA1/neo sequences.

1519. A eukaryotic cell culture transformed with the expression construct of Claim 16, wherein the transformed eukaryotic cell culture is capable of expressing mouse melanocyte stimulating hormone receptor.

20. A eukaryotic cell culture transformed with the expression construct of Claim 17, wherein the transformed eukaryotic cell culture is capable of expressing the human melanocyte stimulating hormone receptor.

21. A method of screening a compound as an inhibitor of agonist binding to a mammalian melanocyte stimulating hormone receptor, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as in Claim 15 capable of expressing the melanocyte stimulating hormone receptor in a eukaryotic cell; and  
25 (b) assaying for ability of the compound to inhibit the binding of a detectable melanocyte stimulating hormone receptor agonist.

22. The method of Claim 21 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

23. The method of Claim 21 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

24. A method of quantitatively detecting a compound as an inhibitor of agonist binding to a mammalian melanocyte stimulating hormone receptor, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as

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in Claim 15 capable of expressing the mammalian melanocyte stimulating hormone receptor in a eukaryotic cell; and

(b) assaying for amount of a compound by measuring the extent of inhibition of binding of a detectable receptor agonist.

525. The method of Claim 24 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

26. The method of Claim 24 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

27. The method of Claim 24 wherein the compound to be tested is present in a human.

28. The method of Claim 24 wherein the compound is present in human blood.

29. The method of Claim 24 wherein the compound is present in human cerebrospinal fluid.

30. The method of Claim 24 wherein the compound is unknown.

1531. An antibody or fragment thereof that is immunologically reactive to a mammalian melanocyte stimulating hormone receptor.

32. The antibody according to Claim 31, wherein the antibody is a monoclonal antibody.

33. The antibody according to Claim 31, wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

34. The antibody according to Claim 31, wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

35. A cell line which produces an antibody or fragment thereof that is immunologically reactive to a mammalian melanocyte stimulating hormone receptor.

2536. The cell line according to Claim 35, wherein the antibody is a monoclonal antibody.

37. The cell line according to Claim 35, wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

38. The cell line according to Claim 35, wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

39. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or fragment thereof according to claim 31 in a pharmaceutically acceptable carrier.

40. An epitope of a mammalian melanocyte stimulating hormone receptor wherein the epitope is immunologically reactive to the antibody or fragment thereof according to claim 31.

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41. The epitope according to claim 40 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

42. The epitope according to claim 40 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

543. A chimeric antibody that is immunologically reactive to a mammalian melanocyte stimulating hormone receptor.

44. The chimeric antibody according to claim 43 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

1045. The chimeric antibody according to claim 43 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

Figure 1A

10 20 30 40 50 60 70  
 TTCCGTGACAA GACTATGTCC ACTCAGGAGC CCCAGAAGAG TCTTCTGGGT TCTCTCACT CCAATGCCAC  
 80 90 100 110 120 130 140  
 CTCTCACCTT GGACTGGCCA CAAACCAAGTC AGAGCCTTGG TGGCTGTATG TGTCCATCCC AGATGGCCTC  
 150 160 170 180 190 200 210  
 TTCCCTCAGCC TAGGGCTGGT GAGTCTGGT GAGAAATGTGC TGGTTGTGAT AGCCATCACCC AAAAACCGCA  
 220 230 240 250 260 270 280  
 ACCTGGACTC CCCCCATGTAT TACTTCATCT GCTGCCCTGGC CCTGTGTGAC CTGATGGTAA GTGTCAAGCAT  
 290 300 310 320 330 340 350  
 CGTGCCTGGAG ACTACTATCA TCCGTGTGCT GGAGGTGGGC ATCCGTGGTGC CCAGAGTGGC TTGGTGCAC  
 360 370 380 390 400 410 420  
 CAGCTGGACA ACCTCATTGA CGTGCCTCATC TGTGGCTCCA TGGTGTCCAG TCTCTGCTTC CTGGGCATCA  
 430 440 450 460 470 480 490  
 TTGCTATAGA CCGCTACATC TCCATCTTCT ATGGGCTGGC TTATCACAGG ATCGTGACGC TGGCCAGAGC  
 500 510 520 530 540 550 560  
 ACCACGGGGT GTCTGGGGCA TCTGGATGGT CAGGATCGTC TCCAGGACCC TCTTATCAC CTACTACAAG  
 570 580 590 600 610 620 630  
 CACACAGGGG TTCTGCTCTG CCTCCTCACT TTCTTCTAG CCATGCTGGC ACTCATGGG ATTCTGTATG  
 640 650 660 670 680 690 700  
 CCCACATGTT CACCGAGACCC TGCCACCACG TCCAGGGCAT TGGCCAGCTC CACAAAAGGC GGGGGTCCAT  
 710 720 730 740 750 760 770  
 CGCCCAAGGC TTCTGCTCTA AGGGTGTGCC CACCCCTACT ATCCCTCTGG GGATTTTCTT CCTGTGTGCG  
 780 790 800 810 820 830 840  
 GGGCCCTTCT TCCCTGCATCT CTTCCTCATC GTCCCTCTGGC CTCAGGACCC CACCTGGCAG TCCATCTCA  
 850 860 870 880 890 900 910  
 AGAACTTCAA CCTCTTCTC CTCCTCATCG TCCTCAGCTC CACTGTGAC CCCCTCACT ATGCTTCCG  
 920 930 940 950 960 970 980  
 CAGCCAGGAG CTCCGCATGA CACTCAAGGA CGTGCCTGCTG TGCTCCGTGAT GATCAGAGGG CGCTGGGCAG  
 990 1000 1010 1020 1030 1040 1050  
 AGGGTACACAG TGATATCCAG TGGCCCTCCAT CTGTGACACC ACAGGTACTC ATCCCTTCTT GATCTCCATT  
 1060 1070 1080 1090 1100 1110 1120  
 TCTCTAAGGC TCCACAGGAT GAGCTTAAA ATAGAAACCC AGAGTGCCTG GGGCCAGGAG AAAGGGTAAC  
 1130 1140 1150 1160 1170 1180 1190  
 TCTGACTGCA GGGCTCACCC AGGGCAGCTA CGGGAACTGG AGGAGACAGG GATGGGAACCT CTAAGCCCTGA  
 1200 1210 1220 1230 1240 1250 1260  
 CCAAGGGTCA GACCACAGGC TCCGTAAAGAG CTTCACCTCT CCCCCACCTAC AGGCAACTCC TCCCTCAAGGC

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Figure 1B

10 20 30 40 50 60 70  
 CCCGCATGTG GCGGCCCTCA ATGGAGGGCT CTGAGAACGA CTITTAACAC GCAGAGAAAA AGCTCCATTC  
 80 90 100 110 120 130 140  
 TTCCCCAGACCC TCAGGGCAGC CCTGGCCCAG GAAGGGCAGGA GACAGAGGCC AGGACGGTCC AGAGGTGTCC  
 150 160 170 180 190 200 210  
 AAAATGTCTG CGAACCTGAG GACCAAGCCAC CAGGGAAAGAC GCAGGGAGGG AGCTGAGGAC GAGGCTTGGT  
 220 230 240 250 260 270 280  
 TGTGAGAACAT CCTGAGCCCA GGCGGTGAT GCCAGGGAGGT GTCTGGACTG GCTGGCCAT GCCTGGCTG  
 290 300 310 320 330 340 350  
 ACCTGTCCAG CCACGGAGAG CGTGTGAGGG CAGATCTGGG CGTGGCCAGA TCGAAGGAGG CAGGCATGGC  
 360 370 380 390 400 410 420  
 GACACCCAAAG GCCCCCTGGC ACCACCATGA ACTAACCGAG ACACCTGGAG GGGAAAGAACT GTGGGGACCT  
 430 440 450 460 470 480 490  
 CGAGGGCTCC AACGACTCTT TCTCTGCTTCC TGGACAGGAC TATGGCTGTG CAGGGATCCC AGAGAAAGACT  
 500 510 520 530 540 550 560  
 TCTGGGCTCC CTCAACTCCA CCCCCCACAGC CATCCCCCAG CTGGGGCTGG CTGCCAACCA GACAGGAGCC  
 570 580 590 600 610 620 630  
 CGCTGCGCTGG AGGTGTCCAT CTCTGACCCGG CTCTTCCCTCA GCCTGGGGCT GGTGAGCTTC GTGGAGAAACC  
 640 650 660 670 680 690 700  
 CGCTGGTGGT GGGCACCATC CCCAAGAACCC GGAAACCTGCA CTCACCCATG TACTGCTTCA TCTGCTGCC  
 710 720 730 740 750 760 770  
 CCCCTTGCTGG GACCTGCTGG TGAGGGGGAC CAACCTGCTG GAGACGGGGG TCATCCTCTT GCTGGAGGCC  
 780 790 800 810 820 830 840  
 CGTGCAGTGG TGGCCCCGGG TGGGGTGTG CAGCAGCTGG ACAATGTCA TGACGTGATC ACCTGGACCT  
 850 860 870 880 890 900 910  
 CCATGCTGTC CAGCCCTGTC TTCTGGGGG CCATCCCCGT GGACCCGTAC ATCTCCATCT TCTACGGACT  
 920 930 940 950 960 970 980  
 CGGCTACCAAC ACCATGGTGA CCCGGGGGGG GGGGGGGGGA CCCCTGGGG CCATCTGGGT GGCCACTGTC  
 990 1000 1010 1020 1030 1040 1050  
 GTCTTCAGCA CGCTCTTCAT CCCCTACTAC GACCAACGTGG CGCTGCTGCT GTGGCTGGT GTCTTCTTCC  
 1060 1070 1080 1090 1100 1110 1120  
 TGGCTATGCT GTGGCTCATG CCCGTGCTGT ACCTCCACAT GCTGGGGGGG GCCTGGCAGG AGGGCCAGGG  
 1130 1140 1150 1160 1170 1180 1190  
 CATGGGGGGG CTCCACAAGA CCCAGGGGGG CGTCCACCAAG CGCTTGGGGG TTAAAGGGCC TGTCAACCTC  
 1200 1210 1220 1230 1240 1250 1260  
 ACCATGCTGG TGGGCATTTT CTTCCTGTC TGGGGGGGGT CTTCCTGCA TCTCACACTC ATCGTCTCT

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Figure 1C

1270        1280        1290        1300        1310        1320        1330  
CCCCCGAGCA CCCCCACGTGCC CGCTGCATCT TCAAGAACTT CAACCTCTTT CTCCCCCTCA TCATCTGCAA  
1340        1350        1360        1370        1380        1390        1400  
TGCCCATCATC GACCCCCCTCA TCTACGGCCTT CCACAGCCAG GAGCTCCGCA GGACGGCTCAA GGAGCTGCTC  
1410        1420        1430        1440        1450        1460        1470  
ACATGCTCCT CGTGAGCCGCC GTGCACCCGCC TTTAAGTGTG CTGGCCAGAG GGAGGTGGTG ATATTGTGGT  
1480        1490        1500        1510        1520        1530        1540  
CTGGTTCCCTG TGTGACCCCTG CCCAGTTCCCT TACCTCCCTG GTCCCCGTTT GTCAAAAGAGG ATGGACTAAA  
1550        1560        1570        1580        1590        1600        1610  
TGATCTCTGA AAGTGTGAA GCGCCGACCC TTCTGGCCAG GGAGGGGTCC TCCAAAACTC CAGGCAGGAC  
1620        1630  
TTCTCACCAAG CAGTCGTGGG AAC

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**Figure 2A**

mouse MSH-R	m	s	t	Q	e	p	Q	k	a	L	v	G	S	L	N	S	n	a	T	s	h	21
human MSH-R	m	a	v	Q	g	s	Q	r	r	L	l	G	S	L	N	S	t	p	T	a	i	21
human ACTH-R																						7
rat cannab.	m	(2-83)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	102
																						1
mouse MSH-R	-	-	L	G	L	A	T	N	Q	s	p	w	C	L	y	v	S	I	P	D	40	
human MSH-R	p	q	L	G	L	A	a	N	Q	t	g	a	r	C	L	e	v	S	I	s	D	42
human ACTH-R	y	e	n	i	n	T	a	r	n	n	s	d	C	p	r	v	v	I	P	e	28	
rat cannab.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	123
																						1
mouse MSH-R	G	L	F	L	S	L	G	L	V	S	L	V	E	N	v	L	V	V	i	A	I	61
human MSH-R	G	L	F	L	S	L	G	L	V	S	L	V	E	N	a	L	V	a	t	I	I	63
human ACTH-R	e	i	f	T	i	s	i	v	g	v	I	E	N	I	i	v	I	I	A	v	v	7
rat cannab.	-	L	-	L	T	L	G	-	-	V	L	E	N	L	L	V	L	-	-	I	-	142

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**Figure 2B**

	II																					
mouse MSH-R	t	K	N	R	N	L	H	c	P	M	Y	y	F	I	C	C	L	A	L	S	D	82
human MSH-R	a	K	N	R	N	L	H	s	P	M	Y	c	F	I	C	C	L	A	L	S	D	84
human ACTH-R	f	K	N	k	N	L	q	a	P	M	Y	f	F	I	C	s	L	A	i	S	D	70
rat cannab.	-	-	-	R	-	L	-	-	P	-	Y	-	F	I	-	S	L	A	-	-	D	163
mouse MSH-R	L	m	y	S	v	s	i	y	L	E	T	t	i	I	L	L	E	v	G	i	103	
human MSH-R	L	L	y	S	g	t	n	v	L	E	T	a	v	I	L	L	E	a	G	a	105	
human ACTH-R	m	L	g	S	I	y	k	i	L	E	n	i	i	I	i	L	r	r	m	G	y	91
rat cannab.	L	L	G	S	V	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	184
mouse MSH-R	L	y	A	R	v	A	l	v	Q	Q	L	D	N	I	I	D	Y	I	i	C	b	124
human MSH-R	L	V	A	R	a	A	v	l	Q	Q	L	D	N	y	I	D	Y	i	t	C	s	126
human ACTH-R	L	k	p	R	g	s	f	e	t	a	D	d	i	I	D	s	I	f	v	I	112	
rat cannab.	-	-	-	-	-	-	-	-	F	-	-	-	-	-	-	-	-	-	V	-	205	

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Figure 2C

	III												IV												
mouse MSH-R	S	M	v	S	S	L	C	F	L	G	i	I	A	i	D	R	Y	I	S	I	I	F	I	145	
human MSH-R	S	M	L	S	S	L	C	F	L	G	a	I	A	v	D	R	Y	I	S	I	I	F	I	147	
human ACTH-R	S	I	L	E	S	i	f	s	L	s	v	I	A	a	D	R	Y	I	S	I	I	F	I	133	
rat cannab.	-	-	-	-	G	S	L	F	-	L	-	Y	-	A	I	D	R	Y	I	S	I	-	-	226	
mouse MSH-R	Y	A	L	R	Y	H	S	I	V	T	L	P	R	A	r	R	A	Y	V	g	v	g	I	166	
human MSH-R	Y	A	L	R	Y	H	S	I	V	T	L	P	R	A	v	a	R	A	Y	a	a	a	I	168	
human ACTH-R	h	A	L	R	Y	H	S	I	V	T	m	r	R	t	v	v	v	I	t	v	v	v	I	154	
rat cannab.	-	-	L	-	Y	-	-	I	V	T	-	P	-	A	v	v	A	-	-	-	-	-	-	247	
mouse MSH-R	W	m	v	S	i	v	s	S	T	L	F	I	t	Y	Y	k	H	t	A	v	L	v	L	187	
human MSH-R	W	v	a	S	v	v	f	S	T	L	F	I	a	Y	d	H	v	A	v	A	v	L	v	L	189
human ACTH-R	W	T	f	c	t	g	t	g	i	t	m	v	i	f	s	h	H	v	p	t	v	v	v	175	
rat cannab.	W	T	-	-	I	V	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	-	v	268	

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Figure 2D

	V																				
mouse MSH-R	L	C	L	Y	t	F	F	L	A	M	L	a	L	M	A	i	L	Y	a	H	
human MSH-R	L	C	L	Y	v	F	F	L	A	M	L	v	L	M	A	v	L	Y	H	M	
human ACTH-R	i	t	f	t	s	l	F	p	l	M	L	v	f	i	i	c	L	Y	v	H	
rat cannab.	-	-	-	-	-	-	F	P	L	-	-	-	-	L	-	-	-	-	-	289	
	V																				
mouse MSH-R	F	t	R	A	C	Q	H	v	Q	G	I	A	q	L	H	K	R	Q	R	229	
human MSH-R	L	a	R	A	C	Q	H	a	Q	G	I	A	R	L	H	K	R	Q	R	231	
human ACTH-R	F	-	-	-	-	-	-	-	-	l	1	A	R	s	H	t	R	k	i	210	
rat cannab.	-	-	-	-	-	-	-	-	-	(31)	-	-	-	-	-	R	P	-	-	338	
	VI																				
mouse MSH-R	Q	G	F	s	L	K	G	A	a	T	L	T	I	L	G	I	F	L	C	250	
human MSH-R	Q	G	F	g	L	K	G	A	v	T	L	T	I	L	G	I	F	F	L	C	252
human ACTH-R	p	r	a	n	m	K	G	A	i	T	L	T	I	L	G	v	F	i	f	C	231
rat cannab.	-	R	-	-	-	A	-	T	L	-	-	L	-	V	-	I	-	C	-	359	

SUBSTITUTE SHEET

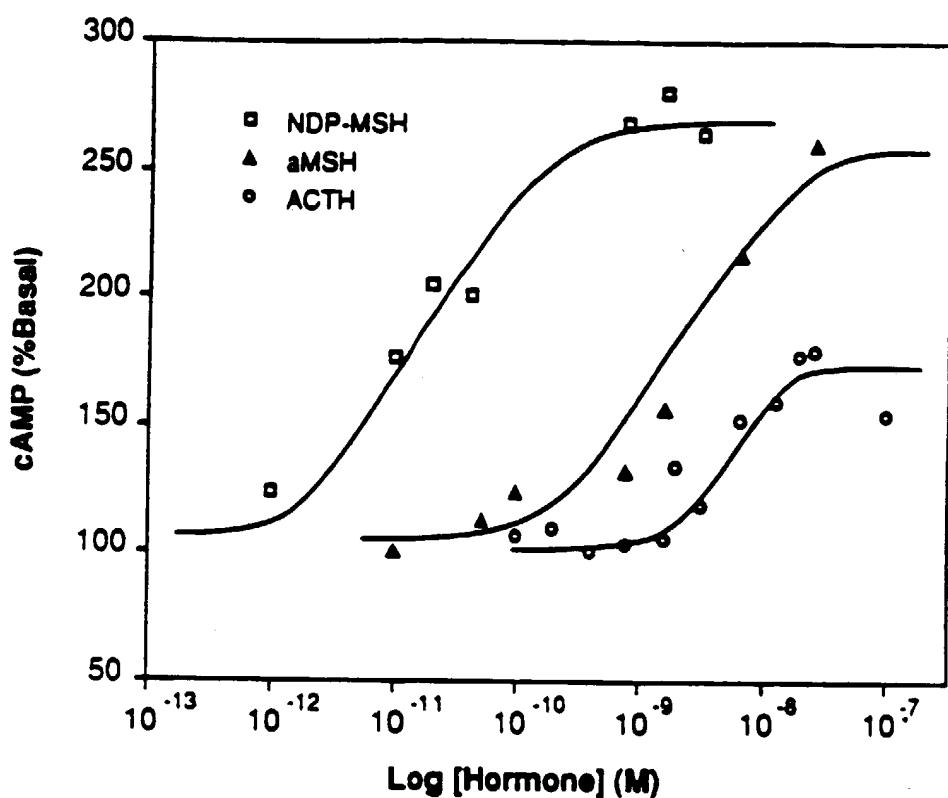
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Figure 2E

	VI												VII												
mouse MSH-R	W	G	P	F	F	H	L	L	I	Y	L	C	P	q	H	P	T	C	s					271	
human MSH-R	W	G	P	F	F	H	L	t	L	I	Y	L	C	P	e	H	P	T	C	g					273
human ACTH-R	W	a	P	F	F	v	L	H	v	L	m	t	f	C	P	s	n	P	y	C	a				252
rat cannab.	W	P	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	380	
mouse MSH-R	C	I	F	K	N	F	N	L	F	L	I	L	I	v	I	s	s	t	v	D	P	L		292	
human MSH-R	C	I	F	K	N	F	N	L	F	L	a	L	I	i	C	N	A	i	I	D	P	L		294	
human ACTH-R	C	y	m	s	I	F	q	v	n	g	M	L	I	m	C	N	A	v	I	I	D	P	f	273	
rat cannab.	-	I	-	-	F	-	-	-	-	M	L	-	-	M	L	-	L	N	S	T	V	-	P	-	401
mouse MSH-R	I	Y	A	F	R	S	Q	E	L	R	m	T	L	K	E	V	L	I	C	S	--	W		317	
human MSH-R	I	Y	A	F	h	S	Q	E	L	R	r	T	L	K	e	V	L	t	C	S	--	W		316	
human ACTH-R	I	Y	A	F	R	S	P	E	L	R	d	a	f	K	m	i	f	C	S	ry	W		297		
rat cannab.	I	Y	A	-	R	S	-	-	L	R	-	A	F	-	M	-	F	-	M	-	-	S	--(56)	483	

SUBSTITUTE SHEET

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Figure 3

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Fig. 4

adrenal	adrenal	pituitary	liver (1)	liver (2)	lung	thyroid	kidney	melanoma (2)	melanoma (1)
9.49-									
7.46-									
4.40-									
2.37-									
1.35-									

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Figure 4A

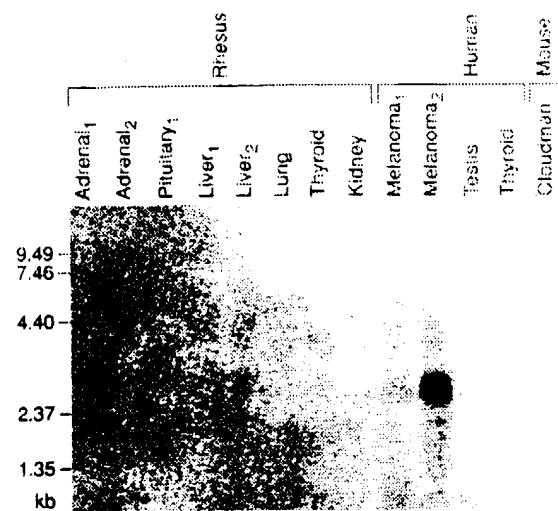


Figure 4B



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## INTERNATIONAL SEARCH REPORT

PCT/US 93/03247

International Application No.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) <sup>9</sup>			
According to International Patent Classification (IPC) or to both National Classification and IPC			
Int.Cl. 5 C12N15/12; C12N15/62;	C07K13/00; A61K37/02;	C12P21/08; A61K39/395;	C12N5/10 C12Q1/68
II. FIELDS SEARCHED			
Minimum Documentation Searched <sup>7</sup>			
Classification System		Classification Symbols	
Int.Cl. 5	C12N ;	C07K ;	A61K ;
G01N			
Documentance Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>			
III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>10</sup>			
Category <sup>11</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>		Relevant to Claim No. <sup>13</sup>
P, X	<b>SCIENCE</b> vol. 257, 28 August 1992, LANCASTER, PA pages 1248 - 1251 Mountjoy KG; Robbins LS; Mortrud MT; Cone RD; 'The cloning of a family of genes that encode the melanocortin receptors.' see the whole document ---  <b>FEBS LETTERS.</b> vol. 309, no. 3, 14 September 1992, AMSTERDAM NL pages 417 - 420 Chhajlani V; Wikberg JE 'Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA.' see the whole document ---  --- ---		1-45
P, X			1-45
			-/-
* Special categories of cited documents : <sup>10</sup> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "T" document published prior to the international filing date but later than the priority date claimed			
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu- ments, such combination being obvious to a person skilled in the art "A" document member of the same patent family			
IV. CERTIFICATION			
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report		
15 SEPTEMBER 1993	15 SEPTEMBER 1993		
International Searching Authority	Signature of Authorized Officer		
EUROPEAN PATENT OFFICE	NAUCHE S.A.		

III. DOCUMENTS CONSIDERED TO BE RELEVANT		(CONTINUED FROM THE SECOND SHEET)
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	<p>THE BIOCHEMICAL JOURNAL      vol. 286, 1 September 1992, LONDON, GB      pages 377 - 382      Ahmed AR; Olivier GW; Adams G; Erskine ME; Kinsman RG; Branch SK; Moss SH; Notarianni LJ; Pouton CW; 'Isolation and partial purification of a melanocyte-stimulating hormone receptor from B16 murine melanoma cells. A novel approach using a cleavable biotinylated photoactivated ligand and streptavidin-coated magnetic beads.'</p> <p>see the whole document</p> <p>---</p>	1-45
A	<p>JOURNAL OF CELLULAR PHYSIOLOGY      vol. 137, no. 1, October 1988, WILEY-LISS, INC.      pages 35 - 44      Kameyama K; Montague PM; Hearing VJ; 'Expression of melanocyte stimulating hormone receptors correlates with mammalian pigmentation, and can be modulated by interferons.'</p> <p>see the whole document</p> <p>---</p>	1-45
A	<p>EUROPEAN JOURNAL OF PHARMACOLOGY      vol. 181, no. 1-2, 31 May 1990,      pages 71 - 82      Leiba H; Garty NB; Schmidt-Sole J; Piterman O; Azrad A; Salomon Y; 'The melanocortin receptor in the rat lacrimal gland: a model system for the study of MSH (melanocyte stimulating hormone) as a potential neurotransmitter.'</p> <p>see the whole document</p> <p>-----</p>	1-45